Immunity
Review

Chemokines and Chemokine Receptors: Standing at the Crossroads of Immunobiology and Neurobiology

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There are several molecular entities common to the immune and nervous systems. Salient among them are the chemokines and their receptors, which play remarkably varied and potent roles in immunobiology and neurobioloogy. This review limns several illustrative examples and presents general principles of chemokine action that are manifest in both systems. These include the following: (1) chemokines tend equally to arrest cells and to make them move, in the process of positioning target cells with spatiotemporal precision; (2) signaling and nonsignaling receptors collaborate to adjust the chemokine environment for maximal efficacy; and (3) expression of a single chemokine receptor on circulating blood cells and parenchymal cells is often used to coordinate complex tissue responses. The challenge is to integrate knowledge of the roles of key receptors (as well as their ligands) into a coherent account of events during pathologic processes, in order to guide therapeutic development.

Classification and Organization of the Chemokine System

The immune and nervous systems comprise a number of molecules shared in common. Such findings were not unexpected given the complexity of the immune and nervous systems, but the specific molecules and processes involved have often been fascinatingly surprising. Chemokines and their receptors are prominent examples of joint use by the immune and nervous systems, yet seemed initially to be completely devoted to assisting the function of the immune system.

The chemokine universe is comprised of approximately 50 peptides and 20 receptors in humans, with homologs, orthologs, and related peptides in other vertebrate species (Charo and Ransohoff, 2006; Rot and von Andrian, 2004). Chemokines are divided into families and signal to corresponding families of chemokine receptors (for example, CXC chemokine action is mediated by CXC chemokine receptors). Chemokine receptors are G protein-coupled receptors (GPCRs) and act specifically through pertussis toxin-sensitive $G_{\alpha i}$ components. Chemokine-specific GPCRs are drug targets, and the biotechnology and pharmaceutical industry has mounted substantial efforts to modulate chemokine receptor activity, heightening the medical importance of understanding how chemokines regulate inflammatory disease. First identified by their ability to mediate leukocyte chemoattraction in vitro, chemokines are now recognized to govern a wide array of leukocyte functions during inflammation and immunity.

The numerical mismatch between chemokines and receptors makes it apparent that ligand-receptor relationships may not be simple, and this suspicion has proven to be accurate (Rot and von Andrian, 2004). Several chemokine-receptor pairs are exclusive; for other chemokine receptors, responses can be elicited by as many as 10 individual ligands. Conversely, some chemokines can productively signal to as many as three receptors. There have been several excellent reviews detailing ligand-receptor relationships (Bacon et al., 2002; Zlotnik and Yoshie, 2000; Rot and von Andrian, 2004; Bonecchi et al., 2009).

Chemokines deliver diverse and context-specific signals. The remarkable versatility and functional flexibility of the chemokine system is conferred by (1) their large number; (2) their tightly regulated transcriptional expression; (3) their ability to interact with binding moieties (such as glycosaminoglycans or nonsignaling receptors) after secretion; and (4) their proteolytic processing (Ransohoff, 2003). Lending further plasticity for regulated expression, chemokine genes can be subject to copy-number polymorphism. Chemokine expression, particularly during inflammation, is primarily regulated by inducible transcription, followed by translation, secretion, and turnover. However, two chemokines (CXCL16; CX_3CL1) are expressed as transmembrane components that are regulated by cleavage by members of the ADAM (a disintegrin and metalloprotease) family enzymes, at least for action at a distance as soluble chemoattractants. Membrane-bound CX_3CL1 and CXCL16 can also serve as adhesion molecules for receptor-bearing cells.

The chemokine ligand super-family is further partitioned into subgroups of the largest (CC chemokines; 28 members) and second largest (CXC chemokines; 16 members) families. Genomic organization helps to give order to this large superfamily (Colobran et al., 2007). Most human CXC chemokines are encoded at chromosomal location 4q12-21, with the majority of CC chemokine found at 17q11-21, and these loci are often syntenic in other mammalian species. Chemokine subgroup members, encoded in multigene arrays, are functionally related. For example, CXCL9, CXCL10, and CXCL11 are three CXC-family, IFN-γ-inducible chemokines. These chemokines signal to a single receptor CXCR3 (which is regulated by the Th1 cell-associated transcription factor T-bet) and are clustered together separated by at most a few dozen kilobases. A similar array contains eosinophil-attracting eotaxin peptides CCL24 and

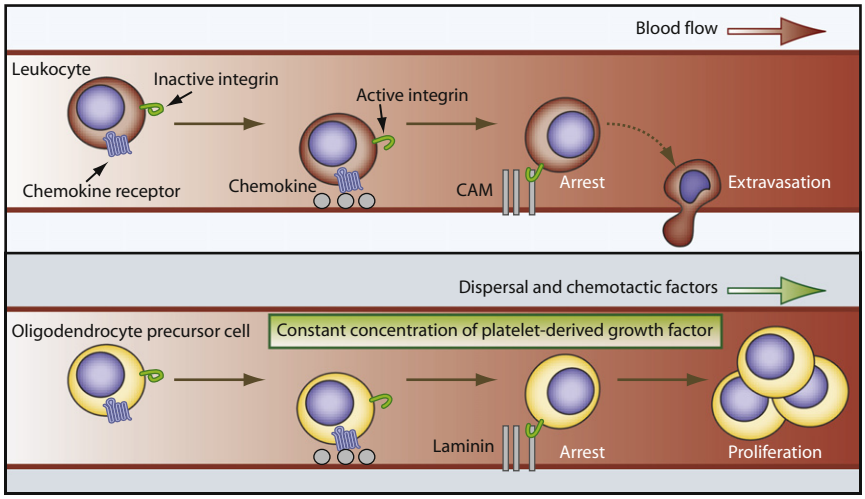

Figure 1. Chemokine Receptor Signaling Mediates Arrest of Moving Cells in the Immune and Nervous Systems

The cartoon illustrates the function of chemokine receptor signaling in the early phases of leukocyte extravasation under flow. Initial leukocyte-endothelial interactions are loose and reversible. Chemokines are immobilized in the vascular lumen by interactions with glycosaminoglycans or by presentation through molecules such as the Duffy antigen receptor for chemokines (DARCs). Chemokine receptor signaling converts inactive integrin to active integrin, capable of high-affinity interaction with cell-adhesion molecules (CAMs) on the endothelial luminal surface. This interaction arrests the leukocyte under flow and extravasation of the leukocyte often follows. The lower panel shows an oligodendrocyte precursor cell (OPC) moving through the presumptive white matter of the postnatal rodent spinal cord. Chemokine CXCL1 is expressed focally in these tissues and signals to CXCR2 on the OPC. In an in vitro model, signaling through CXCR2 leads to increased interaction between the OPC and a laminin substrate. Studies with purified OPCs in vitro, and in vivo analysis of *Cxcr2*$^{-/-}$ mice, suggest that arrest of the OPC in a high local concentration of CXCL1 and PDGF (which is present throughout the developing spinal cord) drives a burst of OPC proliferation.

CCL26, which are members of the CC family. "Solitary" chemokines, such as CXCL12 at Chr.10q11 and CXCL16 at 17p13 found outside multigene arrays, are noted to be paired exclusively with individual signaling receptors and to have distinct functions. With respect to CXCL9, CXCL10, and CXCL11, even though their genomic, structural, and in vitro functional similarities give an appearance of conspicuous redundancy, careful analysis of cells expressing engineered receptors demonstrated selective signaling pathways suggesting distinct functions (Colvin et al., 2004). This concept was confirmed with the demonstration that CXCL9 played a crucial and nonredundant role in antitumor immunity (Gorbachev et al., 2007). Not surprisingly, the role of CXCR3 in T cell trafficking during Th1 cell immune responses has proven surprisingly subtle and intricate (Koch et al., 2009; Lord et al., 2005; Liu et al., 2005, 2006b). It is accordingly accurate to speak both of redundancy and specificity in the chemokine system (Mantovani, 1999; Charo and Ransohoff, 2006; Rot and von Andrian, 2004).

Chemokines Regulate Cell-Cell Interactions

The eponymous action of chemokines toward responsive cells is gradient-dependent chemoattraction in vitro. It seems intuitively appealing to assign chemokines a role in leukocyte trafficking based purely on their ability to drive gradient-dependent migration; this notion, however, is incomplete. Rather, chemokine action causes cells initially to arrest, rather than move (Figure 1), during the multistep process of leukocyte extravasation (Butcher, 1991) under flow (Alon et al., 2003; Alon and Feigelson, 2002; Cinamon et al., 2001a, 2001b, 2004; Laudanna and Alon, 2006; Schreiber et al., 2007; Ransohoff et al., 2007). Perhaps most importantly, chemokines mediate both clustering and conformational changes of integrins, leading to high-affinity and -avidity integrin interactions with cell adhesion molecules (CAMs) on vascular endothelial cells.

Chemokine-chemokine receptor signaling (or action through closely related chemoattractant receptors) is therefore essential for leukocyte-endothelial recognition, which regulates leukocyte trafficking. Leukocyte extravasation requires multiple chemokine-mediated signals. As noted above, the first signal, delivered by chemokines immobilized on the luminal surface of the endothelial cell, helps convert leukocyte rolling under flow on endothelium into arrest, by inducing Rho GTPase family signaling that causes conformational change and redistribution of leukointegrins (Ward, 2009). These alterations in their shape and distribution are required for firm adhesion of leukocyte integrins (such as LFA-1) to CAMs (such as ICAM-1) on endothelium (Shulman et al., 2009). Additional chemokine signals are implicated in "crawling" of leukocytes across endothelium in search of a suitable locus for extravasation. Finally, chemokines are implicated in extravasation itself through inducing cytoskeletal reorganization and chemotaxis toward abluminal chemokines in inflamed tissues. In one classic example, CCL21 is an arrest receptor for lymphocytes rolling on high endothelial venules (HEV) of peripheral lymph nodes (Stein et al., 2000). In the context of leukocyte trafficking, chemokines and their receptors are grouped as homeostatic (chemokines expressed constitutively in organs such as in lymph nodes and spleen, with receptors on leukocytes homing to those organs) or inflammatory (chemokines induced on-demand at sites of inflammation, with cognate receptors on infiltrating leukocytes) (Charo and Ransohoff, 2006).

As predicted, gene targeting studies showed that inflammatory chemokine receptors such as CCR2 and CXCR2 are essential for responses to a wide variety of infectious and inflammatory challenges (Charo and Ransohoff, 2006). Studies of the homeostatic chemokine receptors such as CCR7 and CXCR5 led to a paradigm refinement (if not shift): in early gene-targeting experiments, CXCR5 (Forster et al., 1996), and later CCR7, were implicated in developmental organogenesis for lymphoid tissues, as well as in lymphocyte homing to lymph nodes (Lipp and Muller, 2003). These striking findings showed chemokine receptors mediated cell migration during development, as well as during inflammatory and immune processes in the postnatal organism.

Nonsignaling Chemokine Receptor-like Molecules

Considerable effort seems to have been devoted to maintenance of appropriate fluid and tissue amounts of chemokines (Figure 2).

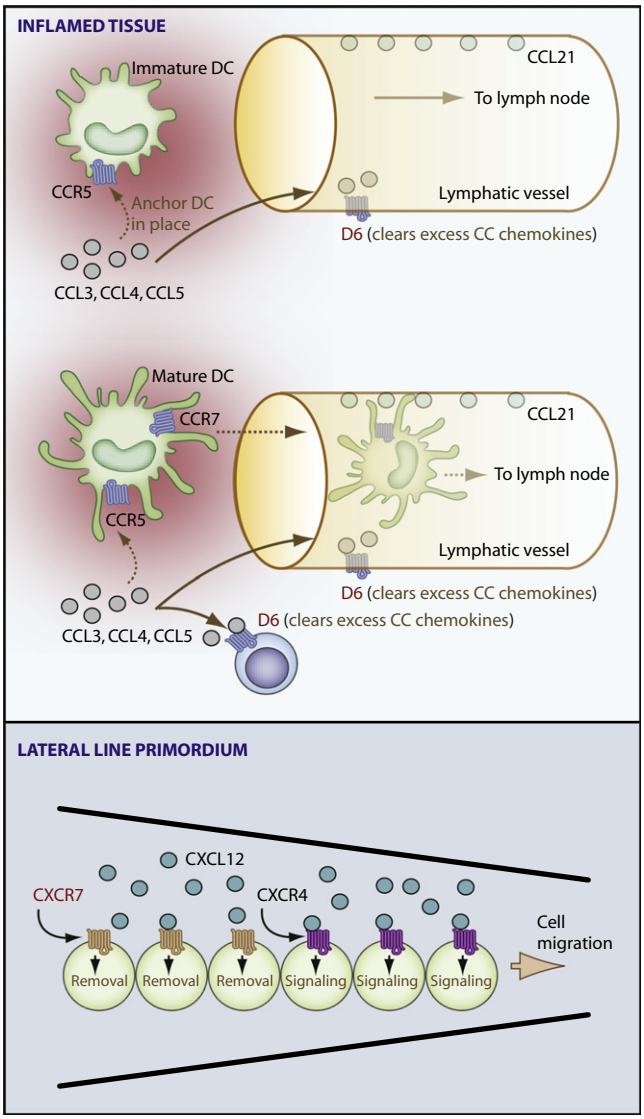

Figure 2. Nonsignaling "Scavenger" Chemokine Receptor-like Molecules Adjust the Chemokine Environment

In inflamed tissues, an immature dendritic cell (DC) encounters a high concentration of chemokines (CCL3, CCL4, and CCL5) that all signal to CCR5 and "anchor" the cell in place while they process antigen and upregulate functions associated with antigen presentation. During this time, D6, a nonsignaling scavenger receptor on lymphatic endothelium, clears excess inflammatory CC chemokines. After maturation, the DC downregulates CCR5, thereby limiting signaling from CCL3, CCL4, and CCL5. The mature DC also upregulates CCR7. At the same time, other leukocytes upregulate D6 and assist in removing these inflammatory chemokines (but not the homeostatic chemokine CCL21) from the environment. The culmination of reduced signaling from inflammatory chemokines and suppressed signaling from inflammatory chemokines favors entry of the DC into the afferent lymphatic vessel, whose lumen is decorated with CCL21. The DC will subsequently traffic to the draining lymph node. As shown on the bottom, the lateral line primordium comprises a population of cells, organized in a coherent linear aggregate, whose migration is mediated by chemokine CXCL12. Cells at the leading edge express the signaling receptor CXCR4. Cells at the trailing edge express CXCR7 and enhance gradient steepness by removing CXCL12.

For example, there are several chemokine receptor-like molecules (D6; duffy antigen receptor for chemokines: DARC; CCX-CKR; CCRL2; and possibly CXCR7) whose primary function seems to be adjusting ambient concentrations of their chemokine ligands (Locati et al., 2005; Mantovani et al., 2006). In some cases, these receptor-like molecules do not signal by the canonical GPCR pathways but rather internalize chemokines either to translocate them elsewhere or to dispose of them. From evaluation of chemokine receptor-deficient mice, it is now apparent that the "signaling" receptors similarly modulate the chemokine environment (Cardona et al., 2008; Mantovani and Locati, 2008). This function of chemokine receptors will need to be taken into account, as therapeutic chemokine receptor blockade becomes a more prevalent treatment for disease (Charo and Ransohoff, 2006).

The chemokine receptor-like molecules that lack G protein coupling mediate important functions in chemokine biology (Rot and von Andrian, 2004). By convention, these molecules, which fail to mediate chemoattraction in vitro and do not increase cytoplasmic calcium concentrations, are termed "nonsignaling" receptors, although they clearly couple the presence of their ligands to cellular responses. The nonsignaling receptors are closely related to chemokine receptors, being "seven-spanning" plasma membrane components. The best characterized are DARC and D6, each of which efficiently binds and internalizes numerous chemokines. Expressed on postcapillary venular endothelium, DARC supports leukocyte recruitment to tissues, through binding inflammatory chemokines abluminally, and internalizing them for transcytosis and immobilization on the lumenal aspect of the capillary, to signal to rolling leukocytes (Pruenster et al., 2009). Parenthetically, DARC has also been widely studied for its role in *Plasmodium vivax* invasion of erythrocytes (Horuk et al., 1993). On red cells, DARC has long been proposed to carry out physiological scavenging of the unbound plasma fraction of its approximate dozen of chemokine ligands. This speculation was confirmed with the recent demonstration that posttransfusion pulmonary inflammation is caused in part by the loss of DARC's scavenging activity on banked erythrocytes (Mangalmurti et al., 2009). DARC therefore plays an exquisitely subtle role in inflammation, removing surplus plasma chemokines that could mediate harmful, indiscriminate inflammation, while orchestrating leukocyte entry into tissues harboring pathogens or sites of damage.

Encoded at human 3p21, near a cluster of chemokine receptor genes, D6 binds at least 12 inflammatory CC chemokines, internalizes them, and targets them for degradation, through constitutive recycling between plasma membrane and endocytic vesicles at an extraordinarily rapid rate (Locati et al., 2005). D6 is expressed constitutively on lymphatic endothelium and, inducibly, on leukocytes (Graham and McKimmie, 2006), and the functional importance of D6 on leukocytes versus lymphatic endothelium is an unresolved question (Graham and McKimmie, 2006). Topical phorbol ester or intradermal complete Freund's adjuvant (CFA) caused remarkably enhanced and sustained inflammation of the skin of D6-deficient mice, because of persistently elevated local chemokine concentrations (Martinez de la Torre et al., 2005; Jamieson et al., 2005).

In this context, D6 is a "scavenger" receptor and plays a major anti-inflammatory role. Studies of autoimmune central nervous system (CNS) inflammation frequently involve a disease model termed experimental autoimmune encephalomyelitis (EAE). A surrogate for the inflammatory aspect of the human disorder

multiple sclerosis (MS), EAE is typically generated by immunization with peptide fragments derived from myelin proteins emulsified in powerful adjuvants. Mice thus immunized develop CNS inflammation including hematogenous leukocyte infiltrates accompanied by motor signs about 2 weeks after immunization.

We speculated that D6-deficient mice might show a worsened course of EAE, if chemokine clearance from the CNS were impaired. Unexpectedly, D6-deficient mice did not generate efficient encephalitogenic responses to immunization with MOG_{35-55} peptide-CFA (complete Freund's adjuvant) and were relatively resistant to EAE (Liu et al., 2006a), possibly because dendritic cells (DCs) trying to access lymphatics were trapped in the "hyperinflamed" immunization site. The role of D6 in immunity might therefore be to remove inflammatory chemokines and allow DCs exiting tissue to respond to homeostatic chemokine present on the lymphatic endothelial lumen. It should be emphasized that the D6 story is far from complete: the functional significance of its expression on lymphatic endothelium and leukocytes remains enigmatic, as does its physiological significance in adjusting the chemokine environment in the diverse settings (inflammation, cancer, pregnancy) in which it's been accorded physiological significance (Borroni et al., 2008, 2009; Martinez de la Torre et al., 2007; Nibbs et al., 2007).

A unifying concept from studying DARC and D6, two nonsignaling receptors is that they exert uniquely disparate functions by virtue of their expression on parenchymal or vascular elements, as compared with their expression on circulating or extravasated blood cells. A cardinal example is DARC, where expression on postcapillary venules mediates one set of effects while its presence on erythrocytes mediates separate and distinct functions (Rot, 2005).

Chemokines in Disease Pathogenesis

The CNS is immune privileged by virtue of lacking resident DCs (Galea et al., 2007). Equally important for the preservation of its postmitotic and fragile cells, the CNS possesses attributes that modify inflammation. These characteristics include the blood-brain barrier (BBB) (Bechmann et al., 2007) and a tendency to recruit hematogenous cells very carefully. Cell loss after administration of the excitotoxin kainic acid (KA) involves neuronal necrosis and illustrates this principle: different to such injury in peripheral tissues (where necrotic injury elicits a rapid cellular response dominated by neutrophils), CNS leukocyte infiltrates after KA appear after a delay and consist primarily of mononuclear phagocytes (Bell and Perry, 1995).

If we consider the phenomenon of stringent control over leukocyte entry into the CNS (Engelhardt and Ransohoff, 2005) and also take account of the importance of chemokines for specificity in leukocyte migration, it is not surprising that early efforts in the field of "neurochemokinology" involved analysis of the functions of chemokines and their receptors in leukocyte trafficking to the inflamed CNS. Much of this work utilized the EAE model of autoimmune inflammatory mechanisms, which are believed to be implicated in MS.

Trafficking of some but not all blood-derived leukocyte cell types to the inflamed CNS of animals with EAE and other model conditions have been attributed to specific chemokines and their receptors. Importantly, disruption of chemokine-mediated leukocyte trafficking also alters disease expression (Proudfoot et al., 2008; Dogan and Karpus, 2004). However, major issues remain unresolved. Although T lymphocytes are required for EAE pathogenesis, no chemokine receptor is known to play a nonredundant, essential role for migration of $CD4^+$ T lymphocytes to the CNS during EAE. Below are examples of current knowledge of chemokine receptors that regulate the migration of leukocyte populations to the inflamed CNS of mice with EAE and other model disorders.

Monocytes accumulate in large numbers in the inflamed CNS of mice with EAE and give rise to macrophages that carry out diverse functions including directly damaging myelin and axons, clearance of tissue debris, and production of cytokines. Macrophages are pathogenic in EAE as shown by depletion studies (Brosnan et al., 1981). Monocytes that enter the CNS are $Ly6C^{hi}$ (King et al., 2009) and belong to a monocyte subset that preferentially uses CCR2 for trafficking to inflamed tissues (Geissmann et al., 2003). Even with a potent immunization regimen, $Ccl2^{-/-}$ mice with EAE exhibit extremely mild disease, with near-complete lack of infiltrating monocytes, and minimal demyelination (Huang et al., 2001). CCR2 was identified as the receptor responsible for CCL2 action in EAE (Fife et al., 2000; Izikson et al., 2000). Immunization with thrice higher antigen concentrations than conventionally used drove atypical neutrophilic EAE in $Ccr2^{-/-}$ mice, underlining the role of CCR2 for monocytic infiltrates (Gaupp et al., 2003). Blocking CCR2 with neutralizing antibodies also suppressed EAE, with particular efficacy for relapsing disease (Mahad and Ransohoff, 2003). The clinical importance of this work was suggested by reports of altered concentrations of CCL2 in the CSF of MS patients. Specifically, MS patients exhibited reduced amounts of CSF CCL2, particularly during active disease, whereas most neuroinflammatory diseases (such as viral encephalitis or HIV-associated dementia) feature very high CSF CCL2 concentrations. In vitro studies suggested that CCL2 in the CNS extracellular space (in equilibrium with the CSF), might be consumed by $CCR2^+$ migrating cells (Mahad et al., 2006). By using a variety of additional models of CNS inflammation and injury, it became evident that CCL2 signals to CCR2 to recruit monocytes to the inflamed CNS (Mildner et al., 2007; Ransohoff, 2007).

NK cells mediate regulatory effects in EAE, as suggested more than a decade ago, by the occurrence of greatly worsened EAE in mice treated with depleting NK1.1 antibodies (Zhang et al., 1997). Later experiments demonstrated that CX_3CR1 was essential for recruitment of NK cells (but not NKT cells, T lymphocytes, or monocytes) into EAE tissues (Huang et al., 2006; Shi and Van Kaer, 2006). Mice lacking CX_3CR1 developed EAE of equivalent severity to mice depleted of NK cells by passive immunization with NK1.1 antibodies. Interestingly, individuals with MS had lower numbers of CX_3CR1^+ NK cells than did relevant controls, and there was a relationship between frequency of circulating CX_3CR1^+ NK cells and MS disease activity (Infante-Duarte et al., 2005).

In many circumstances, neutrophils are poorly recruited to the CNS parenchyma and monocytes seem to be preferred. At the early phases of some EAE models, substantial numbers of neutrophils are observed, and there is indirect evidence that they exert a pathogenic function (McColl et al., 1998). $Cxcr2^{-/-}$ mice were relatively refractory to developing EAE and neutrophils were not detected in the CNS of mutant mice after

immunization. Most tellingly, transfer of small numbers of wild-type neutrophils rescued the ability of *Cxcr2*$^{-/-}$ mice to exhibit signs of EAE (Carlson et al., 2008).

Importantly several chemokine system elements studied in the EAE system are homologous in humans and mice. For example, human CXCR2 functions equivalently to the murine ortholog in knockin mice (Mihara et al., 2005). Furthermore, chemokine receptors are drug targets (Charo and Ransohoff, 2006). Therefore, where appropriate, insights from this research might readily be applied to treating human inflammatory CNS diseases such as MS.

Chemokines in Development and Physiology of the Nervous System

Beginning in 1998 (Ma et al., 1998; Zou et al., 1998; Tachibana et al., 1998), it was discovered that mice lacking CXCR4 or its ligand CXCL12 harbored extensive neurodevelopmental defects (Li and Ransohoff, 2008), with prominent malpositioning of neurons of the cerebellum, dentate gyrus, trigeminal ganglia, dorsal root ganglia, and cortical interneurons as well as aberrant initial trajectory of spinal motor axons (Lazarini et al., 2003). Chemokine CXCL1, in the presence of PDGF, also shows stimulated proliferation of a key population of glial cells, the oligodendrocyte progenitor cells (OPCs) (Robinson et al., 1998). It was later found that CXCR2 and its ligand CXCL1 helped determine both positioning and numbers of oligodendrocytes in the developing spinal cord, by acting as an "arrest receptor" and proliferative signal for OPCs (Tsai et al., 2002). These reports about CXCR4's actions toward neurons and the role of CXCR2 in gliogenesis sparked reconsideration of contemporary phylogenetic research, culminating in the proposal that CXC chemokines emerged at the dawn of vertebrate evolution, to pattern the nervous system (Huising et al., 2003).

In retrospect, it seems unsurprising that chemokines are broadly involved in organ patterning. Chemokine receptors had been shown to organize secondary lymphoid organs during development (Forster et al., 1996). Further, chemokines and chemokine receptors can be identified in the most primitive vertebrates, the agnathous fish *Eptatretus burgeri* (hagfish), but not in invertebrates (DeVries et al., 2006).

Despite their function during development, it seems somewhat perplexing that chemokine receptors are expressed on adult neural cells and mediate physiological or repair functions in the adult CNS (Imitola et al., 2004; Tran and Miller, 2003). However, by analogy with the neurotrophins (Arevalo and Chao, 2005; Chao et al., 2006; Zampieri and Chao, 2006), it seems that chemokines and their receptors play selected roles in nervous system development and are then retained for distinct purposes during postnatal and adult life. A first workshop on "Chemokines and Chemokine Receptors in the Nervous System" (Trettel et al., 2008) was held and addressed this topic among many others.

The most powerful means to localize a receptor in tissue is through immunohistochemistry, which simultaneously reports the presence of protein, its cell of origin, and its subcellular localization. Sadly, production of specific and sensitive antibodies to chemokine receptors has proven difficult. As a result, many preliminary reports using immunohistochemistry to analyze chemokine receptor expression by CNS cells could not be confirmed by definitive studies that included evaluation of wild-type and gene-deficient mice. Standards for CNS tissue immunohistochemistry have been proposed and it would be prudent to regard reports using immunohistochemistry to describe chemokine receptors in CNS tissues as preliminary until all criteria enunciated therein have been fulfilled (Saper and Sawchenko, 2003).

In several cases, chemokine receptors can be found both on circulating leukocytes and on parenchymal cells of the CNS, including neuroepithelial cells. In such cases, four of which are illustrated below, potentially decisive roles are played by these receptors and their ligands in neuroinflammatory processes. This attribute makes these chemokine-receptor pairs particularly worthy of attention for neuroimmunologists and neuroinflammation researchers.

CXCR4 has been termed the "ancestral chemokine receptor" because its homologs are most readily identified in phylogenetic studies of chemokine receptors. The unique ligand for CXCR4 is CXCL12, and the pair was considered monogamous until recent reports that characterized RDC1 (an orphan GPCR) as CXCR7, the second CXCL12 receptor (Sierro et al., 2007; Burns et al., 2006).

Along with showing the earliest expression of any chemokine system elements during embryogenesis, the CXCR4-CXCL12 signaling pair has a large array of developmental functions. These properties seem most pertinent for development of the cardiovascular, hematopoietic, nervous, and urogenital systems, all of which show abnormalities in the mice. During adult life, CXCR4 is expressed on circulating lymphocytes, neutrophils, and monocytes. CXCR4 does not seem, however, to regulate leukocyte accumulation in tissues but does play a role in lymphocyte trafficking within lymph nodes (Okada et al., 2002). Interestingly, distribution of cells within inflamed CNS lesions is in part modulated by interactions between CXCL12, which is normally immobilized at the abluminal surface of cerebral microvessels, and CXCR4 on leukocytes, which are thereby retained in perivascular spaces (McCandless et al., 2006, 2008b). There is preliminary evidence that these observations might be pertinent for human disease (Moll et al., 2009; McCandless et al., 2008a).

Studies of CXCR4 in zebrafish nervous system development provided results that exemplified how decoy receptors might shape chemokine gradients to optimize migratory competence. During zebrafish embryogenesis, approximately 100 migrating cells constitute the primordium that will give rise to posterior lateral line neurons that sense water currents. These cells migrate with anterior-posterior directionality, in a uniform concentration of SDF1a, a CXCL12 homolog. Cells at the leading edge of the migrating cell band express CXCR4b, whereas those at the trailing border express CXCR7. Antisense-mediated knockdown of either CXCR4b or CXCR7 partially impairs migration. Suppression of the CXCL12a ligand, or of both receptors, produces marked defects in migration, halting the primordium. One interpretation (Dambly-Chaudiere et al., 2007) of these findings is that CXCR4b is the signaling receptor for migration, whereas CXCR7 internalizes ligand and establishes the chemokine gradient (Figure 2; Tiveron and Cremer, 2008). These findings illustrate a principle of chemokine action: that nonsignaling receptors can assist in generating chemokine gradients for migration. Applications of these insights to mammalian neurochemokinology are eagerly awaited.

The most obvious adult function of CXCR4 seems related to its expression on a large proportion of tissue stem cells. For example, mobilization of $CD34^+$ hematopoietic stem cells from bone marrow stores can be mediated directly by pharmacological blockade of CXCR4 with AMD3100, one of two FDA-approved drugs targeting chemokine receptors. In the postnatal CNS, CXCR4 functions include modulating GABAergic inputs to dentate gyrus neural progenitor cells, thereby providing regulatory control over proliferation during adult neurogenesis (Bhattacharyya et al., 2008; Kolodziej et al., 2008). These two functions could not be more dissimilar and incarnate the diverse functions of chemokines and chemokine receptors in the nervous and immune systems.

A final neurobiological function of postnatal CXCR4-CXCL12 concerns two relatively new concepts: first, that of gliotransmitters, by which astrocytes participate actively in synaptic transmission (Chen et al., 2006); and second that cytokine TNFα also functions physiologically at CNS synapses (Stellwagen and Malenka, 2006). In an elegant series of studies (Bezzi et al., 2001; Cali et al., 2008), it has been shown that CXCL12 signaling to CXCR4 modulates astrocyte exocytosis of the excitatory neurotransmitter glutamate and that subsequent signaling elicits rapid release of TNFα. This mechanism cleverly uses available materials to support normal synaptic physiology but carries hidden dangers (Allen and Attwell, 2001). If nearby microglia that also express CXCR4 and TNF receptors, respond to these products with augmented TNF release, neuronal damage can ensue, both because of direct toxic effects of TNF and by virtue of impaired astrocyte uptake of glutamate which, present in excess, can engage a neurotoxic process termed excitotoxicity.

Chemokines received their initial attention because their index member CXCL8-IL8 was the first leukocyte chemoattractant that could signal to neutrophils and not to monocytes, unlike classical chemoattractants such as the complement anaphylatoxin C5a (Ransohoff, 2005). One neutrophil receptor for CXCL8, designated CXCR2, was among the first to be cloned and deleted from the murine genome (Cacalano et al., 1994). Of the CXCR2 ligands, CXCL1 (also known as GRO-alpha) has the most extensively documented biology. This peptide has been discovered and rediscovered in contexts as varied as inflammation (Reutershan, 2006), cancer biology (Anisowicz et al., 1987; Luan et al., 1997; Acosta et al., 2008), cell proliferation (Cochran et al., 1983), wound healing (Martins-Green and Hanafusa, 1997), angiogenesis (Strieter et al., 2004), and neuroglial cell biology (Tran and Miller, 2003).

The exceptional versatility of CXCR2 is best shown through its implication in cutaneous wound healing (Devalaraja et al., 2000; Milatovic et al., 2003). When full-thickness skin lesions were characterized in *Cxcr2*$^{-/-}$ mice, the data suggested that CXCR2 was responsible for (1) attracting neutrophils into the skin breach; (2) promoting angiogenesis through expression on angioblasts; and (3) fostering epithelialization via its presence on keratinocytes (Zaja-Milatovic and Richmond, 2008). This concerted action toward mesenchymal, hematopoietic, and epithelial cells during a single tissue repair process lasting only several days is unprecedented in chemokine biology.

Initial studies of oligodendroglial progenitors, purified from neonatal rodent spinal cord, showed that CXCL1, although devoid of intrinsic mitogenic properties, could synergize with platelet-derived growth factor (PDGF) to drive a 4-fold increased proliferative response as compared with PDGF alone (Robinson et al., 1998). Further evaluation in immediately postnatal mice revealed that migrating oligodendrocyte progenitor cells (OPCs) encountered a locally elevated concentration of CXCL1 in the terrain destined to give rise to ventral spinal cord white matter. A combination of assays including in vitro migration studies and slice culture preparations showed that CXCL1 enhanced the interactions between migrating OPCs and the tissue substrate, leading to stalled movement (Figure 1). At the site of arrest, OPCs found themselves in high concentrations of both CXCL1 and PDGF, proliferated vigorously, then differentiated and myelinated axons (Tsai et al., 2002; Tran and Miller, 2003). Ventral CXCL1 expression receded during the first postnatal week, to be succeeded by dorsal CXCL1 production, which promoted myelination of this portion of the spinal cord during the second postnatal week. Mice lacking CXCR2 exhibited a somewhat predictable phenotype: migrating OPCs failed to arrest in the presumptive white matter and accumulated at the tissue edge, the pial surface of the developing spinal cord. Because these misplaced OPCs found axons in a dysregulated sequence, early myelin formation was shifted from the white matter toward the pial surface, and the normal G-ratio, which expresses the tight relationship between axon caliber and myelin thickness, was perturbed (Padovani-Claudio et al., 2006).

As noted above, the presence of $CXCR2^+$ neutrophils was sufficient to render *Ccxr2*$^{-/-}$ mice susceptible to EAE (Carlson et al., 2008). Notably, however, resultant disease was mild, raising the question whether other effects of CXCL1 and CXCR2 might be involved in disease severity (Omari et al., 2009). The components of the CXCR2-CXCL1 signaling system are present in the CNS of mice with EAE and humans with MS (Omari et al., 2005, 2006; Glabinski et al., 1997). Furthermore, an excess of CXCL1 in transgenic mice appears to modulate disease, although the mechanism for this effect remains uncertain (Omari et al., 2009). Further studies of genetic and disease models may help to clarify the specific functions of CXCR2 and its ligands during inflammatory demyelination and other disease states.

CX_3CR1 and Fractalkine: Location, Location, Location

CX_3CL1 (also known as fractalkine) has been fascinating and frustrating researchers since its first description (Schall, 1997). Fractalkine is an unusual chemokine, one of two expressed as a primary single-pass transmembrane molecule that is capable of mediating firm monocyte-endothelial adhesion under flow, or chemoattraction, after proteolytic release. Fractalkine's function toward circulating monocytes has become increasingly clear because of convergent lines of investigation. Mutant mice (*Cx3cr1*$^{-/-}$) were shown to have lessened atherosclerosis because of reduced monocyte infiltration of atheromata, in relevant models (Charo, 2001; Lesnik et al., 2003). Adding spice to the story, humans (about 30% of the Caucasian population) with a variant form of the receptor that blunted adhesive signaling, also showed delayed atherosclerotic endpoints (McDermott et al., 2001, 2003; Moatti et al., 2001). Another contribution to understanding fractalkine biology came from delineating two subsets of circulating monocytes, one of which expressed low levels of Ly6C but high levels of CX_3CR1 (Geissmann et al., 2003, 2008).

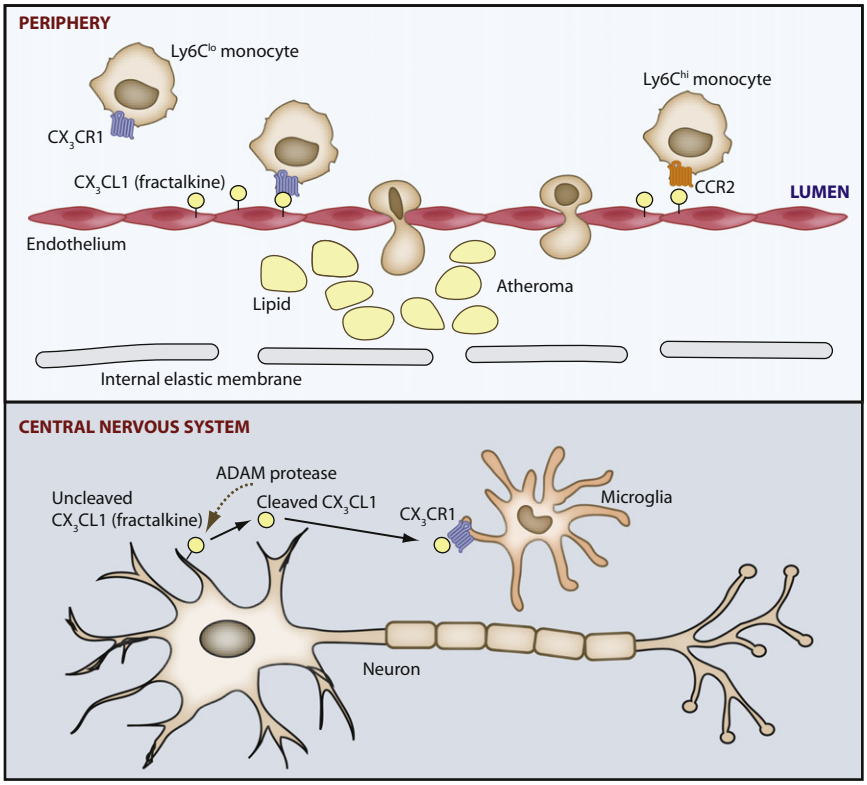

Figure 3. Differential Expression and Function of CX3CL1 in the Peripheral Vasculature and Central nervous System

In the periphery (upper panel), fractalkine is expressed on the endothelium as a single-pass transmembrane component, which can mediate firm arrest under flow. Ly6C^{lo} monocytes express high amounts of CX_3CR1 receptor and can be recruited into a developing atheroma through CX_3CL1-CX_3CR1 interactions. Both *Cx3cr1*$^{-/-}$ mice and individuals harboring a polymorphic variant of CX_3CR1, *CX3CR1*$^{I249/M280}$, which acts as a dominant negative inhibitor of signaling, are relatively protected from atherosclerosis. Atheromata are also infiltrated by Ly6C^{hi} monocytes via signaling to CCR2. As shown in the bottom panel, fractalkine is not present on CNS vessels. Instead, fractalkine is expressed by neurons, in which it can be cleaved and released by ADAM proteases, so that the healthy adult mouse brain contains 150–300 pg/mg protein of soluble fractalkine. Microglia uniformly express CX_3CR1 and respond to ligand by modulating effector functions in a manner that modulates toxicity to neurons. Individuals bearing the $CX_3CR1^{I249/M280}$ variant are at increased risk for age-related macular degeneration (AMD), an inflammatory neurodegenerative disorder, possibly because of neurotoxicity mediated by retinal microglia.

The distinct role of CX_3CR1 in the CNS arises from the expression pattern of the ligand (Figure 3). In particular, vascular structures (endothelial or smooth muscle cells) expresses fractalkine except within the CNS (Sunnemark et al., 2005), where fractalkine is produced by neurons (Harrison et al., 1998). Neurons constitutively produce substantial amounts of soluble fractalkine through ongoing transcription, membrane insertion, and proteolytic release (Cardona et al., 2008). The fractalkine receptor CX_3CR1 is present in the CNS only on microglia (Cardona et al., 2006). Evaluation of several pathological processes that did not involve BBB compromise (and therefore did not entail large-scale entry of peripheral leukocytes) showed that signaling through the fractalkine receptor reduced neuronal damage (Cardona et al., 2006). One potential mechanism for this protective effect was demonstrated in vitro: microglia exposed to fractalkine produce adenosine that signals to protect hippocampal neurons from excitotoxic challenge, through the A1 receptor (Lauro et al., 2008). The potential practical relevance of fractalkine's ability to inhibit microglial neurotoxicity was illustrated vividly by increased liability to age-related macular degeneration in individuals expressing the variant form of the receptor (Combadiere et al., 2007; Tuo et al., 2004).

CCR2 and CCL2: Multiple Roles in Pain States

As noted above, CCR2 is expressed by a subpopulation of monocytes that are typified by the presence of high amounts of membrane Ly6C, and CCR2 is required for entry of monocytes into diverse inflammatory sites including the CNS (Charo and Ransohoff, 2006). CCL2 is the principal ligand for trafficking of CCR2$^+$ "inflammatory" monocytes into the nervous system (Mahad et al., 2006). Mice lacking CCR2 and CCL2 were produced in the late 1990s and were both viable and fertile, with their study yielding remarkable insights into their biology (Charo and Peters, 2003; Gu et al., 1999). Subsequent studies showed potentially distinctive roles for CCR2 and the monocytes whose function it governed, in models of Alzheimer's disease and age-related macular degeneration (El et al., 2007; Ambati et al., 2003). *Ccr2*$^{-/-}$ mice were resistant to models of pain (Abbadie et al., 2003) and, remarkably, both inflammatory pain (elicited by injecting irritants) and neuropathic pain (induced by nerve injury) were about equally affected. Initial evaluation of the affected tissues demonstrated upregulation of CCR2 in the dorsal root ganglia (DRG), in the injured nerve and in the inflamed injection site. It seemed plausible that pain pathways were modulated by the presence of CCR2$^+$ inflammatory monocytes and that loss of CCR2 signaling ameliorated pain by dampening macrophage-mediated inflammation. Concordant results were obtained through study of transgenic mice that overexpressed CCL2 in the CNS under control of the astrocyte-specific GFAP promoter (Menetski et al., 2007).

However, the situation became more complex with the surprising demonstration that DRG neurons upregulated both ligand CCL2 and receptor CCR2 in the context of chronic nerve root damage. Furthermore, CCL2 was packaged in synaptic-like vesicles and its action at CCR2 led to pain-promoting responses, including sensitization of the transient receptor potential vanilloid receptor subtype 1 (TRPV1) ion channel (Jung et al., 2008; White et al., 2005; White and Wilson, 2008). Taken together, these results indicate that CCR2 plays a complex, multifarious role in pain states, both through its expected function on inflammatory

monocytes and via its startling and novel properties as an inducible neuromodulatory receptor on DRG neurons (Abbadie, 2005).

Concluding Remarks

From the perspective of neuroinflammation studies, chemokine research has followed a long and complex odyssey beginning with subset-specific chemoattractants, which seemed likely to mediate the cautious recruitment of leukocytes into the CNS and PNS. Along the way, chemokine receptors were discovered on neural cells, and other CNS-resident cells were shown capable of elaborating chemokine ligands. With the recognition that chemokines and their receptors on these diverse cell types can interact during pathological processes, bewildering and tantalizing vistas of cross-system signaling between the CNS and the immune system become apparent. Deciphering this signaling will place researchers in a position to deploy their insights in the service of novel strategies to treat neurological disease.

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